CLAIMS

1. A purified preparation comprising a plurality of TFPI or TFPI analog molecules, wherein less than about 12% of the TFPI or TFPI analog molecules are modified species, wherein the modified species include one or more of the following:

an oxidized TFPI or TFPI analog molecule, as detected by reverse phase chromatography;

a carbamylated TFPI or TFPI analog molecule, as detected by cation exchange chromatography;

a deamidated TFPI or TFPI analog molecule, as detected by a Promega ISOQUANT® kit;

a TFPI or TFPI analog molecule that comprises a cysteine adduct, as determined by amino acid analysis;

aggregated TFPI or TFPI analog molecules, as detected by size exclusion chromatography; and

a misfolded TFPI or TFPI analog molecule, as detected by non-denaturing SDS-polyacrylamide gel electrophoresis.

2. The purified preparation of claim 1 wherein less than about 9% of the TFPI or TFPI analog molecules are oxidized.

3. The purified preparation of claim 1 wherein less than about 3% of the TFPI or TFPI analog molecules are carbamylated.

4. The purified preparation of claim 1 wherein less than about 9% of the TFPI or TFPI analog molecules are deamidated.

5. The purified preparation of claim 1 wherein less than about 2% of the TFPI or TFPI analog molecules comprise a cysteine adduct.

6. The purified preparation of claim 1 wherein less than about 3% of the TFPI or TFPI analog molecules are aggregated.

7. The purified preparation of claim 1 wherein less than about 3% of the TFPI or TFPI analog molecules are misfolded.

8. The purified preparation of claim 1 wherein members of the plurality of TFPI molecules have the amino acid sequence shown in SEQ ID NO:1.

9. The purified preparation of claim 1 wherein the TFPI analog molecules are ala-TFPI molecules.

10. A pharmaceutical formulation comprising a plurality of TFPI or TFPI analog molecules, wherein less than about 12% of the TFPI or TFPI analog molecules are modified species, wherein the modified species include one or more of the following:

an oxidized TFPI or TFPI analog molecule, as detected by reverse phase chromatography;

a carbamylated TFPI or TFPI analog molecule, as detected by cation exchange chromatography;

a deamidated TFPI or TFPI analog molecule, as detected by a Promega ISOQUANT® kit;

a TFPI or TFPI analog molecule that comprises a cysteine adduct, as determined by amino acid analysis;

aggregated TFPI or TFPI analog molecules, as detected by size exclusion chromatography; and

a misfolded TFPI or TFPI analog molecule, as detected by non-denaturing SDS-polyacrylamide gel electrophoresis.

11. The pharmaceutical formulation of claim 10 wherein less than about 3% of the TFPI or TFPI analog molecules are oxidized.

12. The pharmaceutical formulation of claim 10 wherein less than about 3% of the TFPI or TFPI analog molecules are carbamylated.

13. The pharmaceutical formulation of claim 10 wherein less than about 9% of the TFPI or TFPI analog molecules are deamidated.

14. The pharmaceutical formulation of claim 10 wherein less than about 3% of the TFPI or TFPI analog molecules comprise a cysteine adduct.

15. The pharmaceutical formulation of claim 10 wherein less than about 3% of the TFPI or TFPI analog molecules are aggregated.

16. The pharmaceutical formulation of claim 10 wherein less than about 3% of the TFPI or TFPI analog molecules are misfolded.

17. The pharmaceutical formulation of claim 10 wherein members of the plurality of TFPI or TFPI analog molecules are TFPI molecules that have the amino acid sequence shown in SEQ ID NO:1.

18. The pharmaceutical formulation of claim 10 wherein members of the plurality of TFPI or TFPI analog molecules are ala-TFPI molecules.

19. A pharmaceutical formulation comprising:

a plurality of ala-TFPI molecules, wherein less than about 12% of the ala-TFPI molecules are modified species, wherein the modified species include one or more of the following:

an oxidized ala-TFPI molecule, as detected by reverse phase chromatography;

a carbamylated ala-TFPI molecule, as detected by cation exchange chromatography;

a deamidated ala-TFPI molecule, as detected by a Promega ISOQUANT® kit;

an ala-TFPI molecule that comprises a cysteine adduct, as determined by amino acid analysis;

aggregated ala-TFPI molecules, as detected by size exclusion chromatography; and

a misfolded ala-TFPI molecule, as detected by non-denaturing SDS-polyacrylamide gel electrophoresis,

wherein the pharmaceutical formulation comprises 20 mM sodium citrate, 300 mM L-arginine, and 5 mM methionine, pH 5.5.

20. A method of producing purified TFPI or TFPI analog molecules, comprising the steps of:

(1) expressing TFPI or a TFPI analog in a rifampicin-resistant *E. coli* host cell, wherein the TFPI or the TFPI analog is encoded on a plasmid comprising the following elements:

(a) a transcription promoter;

(b) a ribosome binding site adjacent to the reclac transcription promoter;

(c) a nucleotide coding sequence that encodes the TFPI or the TFPI analog adjacent to the ribosome binding site;

(d) a transcription terminator adjacent to the nucleotide coding sequence;

(e) a replicon;

(f) an antibiotic resistance gene; and

(g) a gene encoding an N-terminal methionine-removing enzyme;

(2) isolating inclusion bodies containing the TFPI or the TFPI analog from the *E. coli* host cell;

(3) isolating the TFPI or the TFPI analog from the inclusion bodies to obtain isolated TFPI or TFPI analog;

(4) refolding the isolated TFPI or TFPI analog to form refolded TFPI or TFPI analog;

(5) purifying the refolded TFPI or TFPI analog by SP-Sepharose fast flow chromatography in the presence of Mg^{++} to form a first preparation of purified TFPI or TFPI analog;

(6) concentrating the first preparation of purified TFPI or TFPI analog to form a first concentrated preparation of purified TFPI or TFPI analog;

(7) purifying the first concentrated preparation of purified TFPI or TFPI analog by Q-Sepharose HP chromatography to form a second preparation of purified TFPI or TFPI analog;

(8) purifying the second preparation of purified TFPI or TFPI analog by butyl HIC chromatography to form a third preparation of purified TFPI or TFPI analog;

(9) purifying the third preparation of purified TFPI or TFPI analog by SP-Sepharose HP chromatography to form a fourth preparation of purified TFPI or TFPI analog;

(10) concentrating the fourth preparation of purified TFPI or TFPI analog to form a second concentrated preparation of purified TFPI or TFPI analog molecules, wherein less than about 12% of the TFPI or TFPI analog molecules are modified TFPI or TFPI analog molecules.

21. The method of claim 20 wherein the transcription promoter is a reclac promoter.

22. The method of claim 20 wherein the ribosome binding site is the ribosome binding site from gene 10 of bacteriophage T7.

23. The method of claim 20 wherein the nucleotide coding sequence encodes ala-TFPI.

24. The method of claim 23 wherein the nucleotide coding sequence is SEQ ID NO:44.

25. The method of claim 20 wherein the transcription terminator comprises the nucleotide sequence shown in SEQ ID NO:42.

26. The method of claim 20 wherein the replicon comprises a pBR322 origin of replication.

27. The method of claim 20 wherein the replicon comprises a rop copy number control gene from pBR322.

28. The method of claim 20 wherein the antibiotic resistance gene is streptomycin adenyltransferase.

29. The method of claim 20 wherein the N-terminal methionine-removing enzyme is *E. coli* methionine aminopeptidase.

30. The method of claim 20 wherein the *E. coli* host cell is MON210 (ATCC Accession No. PTA-5564).

31. A method of purifying TFPI or TFPI analog molecules, comprising the steps of:

(1) purifying recombinantly produced TFPI or TFPI analog molecules by SP-Sepharose fast flow chromatography to form a first preparation of purified TFPI or TFPI analog;

(2) concentrating the first preparation of purified TFPI or TFPI analog to form a first concentrated preparation of purified TFPI or TFPI analog;

(3) purifying the first concentrated preparation of purified TFPI or TFPI analog by Q-Sepharose HP chromatography to form a second preparation of purified TFPI or TFPI analog;

(4) purifying the second preparation of purified TFPI or TFPI analog by butyl HIC chromatography to form a third preparation of purified TFPI or TFPI analog;

(5) purifying the third preparation of purified TFPI or TFPI analog by SP-Sepharose HP chromatography to form a fourth preparation of purified TFPI or TFPI analog;

(6) concentrating the fourth preparation of purified TFPI or TFPI analog to form a second concentrated preparation of purified TFPI or TFPI analog molecules, wherein less than about 12% of the TFPI or TFPI analog molecules are modified TFPI or TFPI analog molecules.

32. The method of claim 31 wherein the SP-Sepharose fast flow chromatography is performed in the presence of Mg^{++}.

33. The method of claim 31 wherein the TFPI or TFPI analog molecules are produced in yeast cells.

34. The method of claim 31 wherein the TFPI or TFPI analog molecules are produced in mammalian cells.

35. The method of claim 34 wherein the mammalian cells are CHO cells.

36. The method of claim 34 wherein the mammalian cells are HepG2 cells.

37. The method of claim 34 wherein the mammalian cells are Chang liver cells.

38. The method of claim 34 wherein the mammalian cells are SK hepatoma cells.

39. A method of expressing TFPI or TFPI analog, comprising:

(1) culturing a rifampicin-resistant *E. coli* host cell in a fermentation medium, wherein the *E. coli* host cell comprises a plasmid having the following elements:

(a) a transcription promoter;

(b) a ribosome binding site adjacent to the reclac transcription promoter;

(c) a nucleotide coding sequence that encodes TFPI or TFPI analog adjacent to the ribosome binding site;

(d) a transcription terminator adjacent to the nucleotide coding sequence;

(e) a replicon;

(f) an antibiotic resistance gene; and

(g) a gene encoding an N-terminal methionine-removing enzyme;

wherein one liter of the fermentation medium comprises 41 g dextrose, 2.5 g $(NH_4)_2SO_4$, 4.0 g sodium polyphosphate, 7.0 g K_2SO_4, 1.63 g $MgSO_4 \cdot 7H_2O$, 2.0 g methionine, 2.0 g glycerol, 0.5 mg H_3BO_4, 0.5 g cobalt chloride, 0.13 g $CuSO_4 \cdot 6H_2O$, 54.0 g $FeCl_3 \cdot 6H_2O$, 11.0 g $MnSO_4 \cdot H_2O$, 0.5 g $Na_2MoO_4 \cdot 2H_2O$, 0.02 $NaSeO_3$, 22.0 g $ZnSO_4 \cdot 7H_2O$, 0.01 ml concentrated H_2SO_4, and 0.55 ml UCON antifoam.

40. The method of claim 39 wherein the transcription promoter is a reclac promoter.

41. The method of claim 39 wherein the ribosome binding site is the ribosome binding site from gene 10 of bacteriophage T7.

42. The method of claim 39 wherein the nucleotide coding sequence encodes ala-TFPI.

43. The method of claim 42 wherein the nucleotide coding sequence is SEQ ID NO:44.

44. The method of claim 39 wherein the transcription terminator comprises the nucleotide sequence shown in SEQ ID NO:42.

45. The method of claim 39 wherein the replicon comprises a pBR322 origin of replication.

46. The method of claim 39 wherein the replicon comprises a rop copy number control gene from pBR322.

47. The method of claim 39 wherein the antibiotic resistance gene is streptomycin adenyltransferase.

48. The method of claim 39 wherein the N-terminal methionine-removing enzyme is *E. coli* methionine aminopeptidase.

49. The method of claim 39 wherein the *E. coli* host cell is MON210 (ATCC Accession No. PTA-5564).